

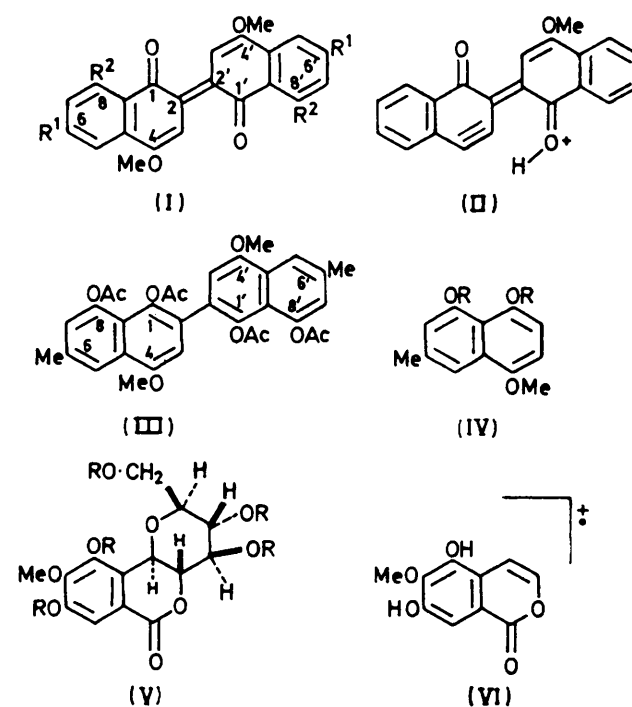
Ebenaceae Extractives. Part IV.¹ Diosindigo A, a Blue Pigment from Several *Diospyros* Species

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The wood of seven of the eighteen species of *Diospyros* examined contains small amounts of a blue pigment, diosindigo A [8,8'-dihydroxy-4,4'-dimethoxy-6,6'-dimethyl-2,2'-binaphthylidene-1,1'-quinone (I; R¹ = Me, R² = OH)]. Other extractives include plumbagin, 7-methyljuglone, isodiospyrin, mamegakinone, bergenin, lupeol, betulin, betulinic acid, oleanolic acid, β -sitosterol, and stigmasterol. Diosindigo A has been synthesised by the oxidation of 4-methoxy-6-methylnaphthalene-1,8-diol, prepared from 7-methyljuglone.

BLUE extracts have been obtained previously² from the wood of several species of *Diospyros*, but the pigments concerned, present in only minute amounts, could not be identified. We have now examined the eighteen South-East Asian species of *Diospyros* listed in the Table and have obtained pale blue or pale green extracts from seven of them. Four of these extracts, from *D. buxifolia*, *D. ehretoides*, *D. Maingayi*, and *D. variegata*, gave isolatable quantities of a sparingly soluble blue pigment and t.l.c. showed that the same compound was present in the other three. The blue compound, C₂₄H₂₀O₆, for which we propose the name 'diosindigo A' was isolated with least difficulty from the wood of *D. buxifolia*, which yielded ca. 5 mg per kg. It behaves as a quinone, being reduced by sodium dithionite or sodium borohydride to give a colourless product which shows u.v. absorption similar to that³ of naphthalene-1,4,5-triol, and forming a leucoacetate, C₃₂H₃₀O₁₀, which exhibits u.v. absorption resembling that⁴ of 2,2'-binaphthyl. The simplicity of its n.m.r. spectrum indicates that the leucoacetate is a symmetrically substituted binaphthyl; only seven signals appear, corresponding to three different aromatic protons and the protons of a methoxy-group, an aromatic methyl group, and two different acetoxy-groups. Diosindigo A has the properties of a bisnaphthaleneindigo, its u.v. and visible light absorption resembling generally that of 4,4'-dimethoxy-2,2'-binaphthylidene-1,1'-quinone (I; R¹ = R² = H).⁵ The two compounds undergo fragmentation in the mass spectrometer in a similar manner, each parent ion losing either a methyl radical or a methoxyl radical from a methoxy-group; the (M - 15)⁺ and (M - 31)⁺ ions are by far the most abundant fragmentation products in each spectrum. The loss of a methoxyl radical is unusual but in the case of the quinone (I; R¹ = R² = H) could give rise to the ion (II) which would be expected to be relatively stable. The formation of a leucoacetate containing four acetoxy-groups implies that diosindigo A contains two hydroxy-groups. These must occupy *peri*-positions, as in (I; R² = OH), because they are strongly hydrogen-bonded to the two carbonyl groups. Thus diosindigo A shows only broad, indefinite i.r. absorption above 1700 cm⁻¹ while the frequency of the quinone carbonyl stretching band (1578 cm⁻¹) is unusually low. All the oxygen functions have now been located and we formulate diosindigo A as

(I; R¹ = Me, R² = OH), the positions of the remaining substituents, the two methyl groups at C-6 and C-6', following from biogenetic considerations.⁶ Diosindigo A leucoacetate therefore has structure (III), in full agreement with the observed n.m.r. spectrum. The latter shows broad signals at δ 8.04 and 7.05 corresponding to



the *meta*-coupled aromatic protons at C-5 and C-5', and at C-7 and C-7', respectively, the signal at lower field being associated with the protons *peri* to the methoxy-groups; the remaining aromatic protons at C-3 and C-3' give rise to a sharp singlet at δ 6.72. The signals for the protons of the methoxy-groups at C-4 and C-4' (δ 3.98), the methyl groups at C-6 and C-6' (δ 2.53), and the acetoxy-groups at C-8 and C-8' (δ 2.36) have the expected chemical shifts but the signal for the C-1 and C-1' acetoxy-protons (δ 2.00) appears at higher field than usual, no doubt because of the shielding effects of the aryl groups at C-2 and C-2'.

We confirmed the structure of diosindigo A by synthe-

¹ Part III, A. Fallas and R. H. Thomson, *J. Chem. Soc. (C)*, 1968, 2279.

² A. G. Brown, A. Fallas, J. C. Lovic, A. Weddell, and R. H. Thomson, unpublished work in this Department.

³ C. Daglish, *J. Amer. Chem. Soc.*, 1950, **72**, 4859.

⁴ R. A. Friedel, M. Orchin, and L. Reggel, *J. Amer. Chem. Soc.*, 1948, **70**, 199.

⁵ S. Goldschmidt and H. Wessbecher, *Ber.*, 1928, **61**, 372.

⁶ A. J. Birch and F. W. Donovan, *Chem. and Ind.*, 1954, 1047.

sising it from 7-methyljuglone (5-hydroxy-7-methyl-1,4-naphthoquinone). 1,4-Naphthoquinone itself is known⁷ to undergo reduction and monomethylation on treatment with tin(II) chloride and phosphoryl chloride in methanol with the formation of 4-methoxy-1-naphthol. A similar reaction with 7-methyljuglone gave a methoxynaphthol which was isolated as the corresponding diacetate (IV; R = Ac). The position of the methoxy-group in this compound follows from the resemblance of its n.m.r. spectrum to that of 4,5-diacetoxy-1-methoxynaphthalene, which can be prepared in a similar manner from juglone and whose structure we have established unequivocally.⁸ Alkaline hydrolysis of the diacetate (IV; R = Ac) and treatment of a solution of the resulting methoxynaphthol (IV; R = H) in chloroform with lead(IV) oxide resulted in coupling at the least-hindered position (C-3); subsequent oxidation of the product gave the binaphthylquinone (I; R¹ = Me, R² = OH) which was identical with diosindigo A. If air is bubbled through a solution of the methoxynaphthol (IV; R = H) in chloroform, diosindigo A is formed quantitatively,

Extractives from woods of *Diospyros* species

Species	Dios-indigo A	Lupeol	Betulonic acid	Betulinic acid	β-Sitosterol/Stigmasterol*	Oleanolic/Ursolic acid*
<i>D. buxifolia</i> (Blume) Hiern	+	+	+	+	+	
<i>D. castanea</i> (Craib) Fletcher		+	+	+	+	+
<i>D. cauliflora</i> Blume	+	+	+	+	+	+
<i>D. Curranii</i> Merrill	+	+	+	+	+	+
<i>D. Diepenhorstii</i> Miq.		+		+	+	
<i>D. ehretoides</i> Wall.†	+	+		+		
<i>D. elliptifolia</i> Merrill		+	+	+		
<i>D. euvana</i> Bakh.		+	+	+	+	+
<i>D. Maingayi</i> (Hiern) Bakh.‡	+	+	+	+		
<i>D. mollis</i> Griff.		+	+		+	
<i>D. montana</i> Roxb.		+	+	+	+	+
<i>D. pseudo-malabarica</i> Bakh.		+	+	+		
<i>D. Sanza-Minika</i> A. Chevalier†		+	+	+	+	
<i>D. rhodocalyx</i> Kurz.		+				
<i>D. siamensis</i> Hochr.		+	+	+		
<i>D. sumatrana</i> Miq.	+	+	+		+	
<i>D. Toposia</i> Buch.-Ham.		+				
<i>D. variegata</i> Kurz.	+	+	+	+	+	

* Not separable by t.l.c. under the conditions used. † For other extractives, see Experimental section. ‡ Diosindigo A (3 mg) was isolated from this wood (1.8 kg).

suggesting that the natural pigment might be an artefact formed during the extraction and purification operations. However when we submitted either the methoxynaphthol or the reduced form of diosindigo A to the isolation procedure only a trace of diosindigo A was obtained and most

of the starting materials were recovered. We conclude that the pigment itself is present in the wood. Since our preliminary note⁹ the isolation of diosindigo A has been reported from five Japanese species of *Diospyros*¹⁰ and from three Angolan *Euclea*¹¹ or *Diospyros*¹² species.

The preliminary examination by t.l.c. of the chloroform extracts from the eighteen *Diospyros* species indicated the presence of the triterpenoids and steroids listed in the Table; we confirmed this by isolating these extractives from the four species *D. buxifolia*, *D. variegata*, *D. ehretoides*, and *D. Sanza-Minika*. Acetylation of the mixtures of the triterpenoid acids and of the sterols facilitated their separation. *D. ehretoides* also afforded the quinones plumbagin, 7-methyljuglone, mamegakinone, and isodiospyrin, and the acetone extract from the heartwood of *D. Sanza-Minika* gave a copious supply of bergenin (V; R = H) monohydrate.¹³ This last compound (m.p. 129–131°) on being heated gave anhydrous bergenin (m.p. 232–234°) and on acetylation gave bergenin penta-acetate (V; R = Ac). The mass spectrum of bergenin is particularly simple, the molecular ion undergoing loss of C₄H₈O₄ with the formation of an ion *m/e* 208 [presumably (VI)] as the only abundant fragmentation product.

EXPERIMENTAL

The solvents specified below were used unless otherwise stated. T.l.c. was performed on silica gel (Merck Kieselgel GF₂₅₄) with chloroform for development. I.r. absorption spectra were measured for potassium bromide discs and u.v.-visible absorption spectra for ethanolic solutions with Perkin-Elmer Infracord 237 and 137UV spectrophotometers, respectively. N.m.r. spectra were measured for solutions in deuteriochloroform with Varian A60 and HA100 spectrometers (tetramethylsilane as internal standard). Mass spectra were determined on an A.E.I. MS-902 high resolution spectrometer at 70 eV; only the more significant ions are recorded. The identity of a compound with an authentic specimen was usually established by mixed m.p. and by comparisons of i.r., u.v., and visible spectra, t.l.c. or g.l.c. behaviour, and, where appropriate, mass spectra and optical rotations. The homogeneity of the steroids and triterpenoids was established by g.l.c. at 120–200° with a Perkin-Elmer F-11 gas chromatograph fitted with columns packed with silicone gum rubber E301 on Celite.

Preliminary Examination of Extracts from *Diospyros* Species.—The chloroform extract of the finely ground wood (100 g) was separated by t.l.c. into its components, which were identified by comparing their behaviour with that of authentic specimens when subjected to t.l.c. in the three solvent systems, chloroform, chloroform-methanol (4:1), and benzene. The compounds identified are indicated in the Table.

Extraction of Wood of *Diospyros buxifolia* (Blume) Hiern.—Finely ground heartwood and sapwood (1.5 kg) was extracted (Soxhlet) successively with light petroleum (b.p. 60–80°) and with chloroform for 24 h. Evaporation of the combined extracts gave a dark residue (6.5 g) which was

¹¹ M. A. Ferreira, M. Aurea Cruz Costa, A. Correia Alves, and M. H. Lopes, *Phytochemistry*, 1973, **12**, 433; 1974, **13**, 499.

¹² M. A. Ferreira, A. C. Costa, and A. Correia Alves, *Plantes médicinales et phytothérapie*, 1972, **6**, 32.

¹³ J. E. Hay and L. J. Haynes, *J. Chem. Soc.*, 1958, 2231.

⁷ C. M. Smith, U.S.P. 2,572,822/1951 (*Chem. Abs.*, 1952, **46**, 3571b).

⁸ O. C. Musgrave and D. Skoyles, forthcoming publication.

⁹ O. C. Musgrave and D. Skoyles, *Chem. Comm.*, 1970, 1461.

¹⁰ M. Tezuka, C. Takahashi, M. Kuroyanagi, M. Satake, K. Yoshihira, and S. Natori, *Phytochemistry*, 1973, **12**, 175, and earlier papers.

separated by repeated t.l.c. into a green oil, four colourless fractions which on being crystallised from ethanol afforded lupeol (400 mg), β -sitosterol (330 mg), betulin (500 mg), and betulinic acid (1.03 g), respectively, and complex mixtures of unidentified aliphatic materials (2.46 g).

Diosindigo A.—After further t.l.c. and column chromatography on silica gel the green oil yielded *diosindigo A* (8,8'-dihydroxy-4,4'-dimethoxy-6,6'-dimethyl-2,2'-binaphthylidene-1,1'-quinone), which crystallised from light petroleum (b.p. 80–100°)—chloroform in deep blue needles (8 mg), m.p. 317° (decomp.) (Found: *M*, 404.1259. $C_{24}H_{20}O_6$ requires *M*, 404.1260), ν_{\max} 1578 cm^{-1} (hydrogen-bonded quinone C=O), λ_{\max} (CHCl₃) 294 (log ϵ 4.33) and 697 nm (4.45), λ_{infl} 328 (log ϵ 4.10), 520 (3.69), and 657 nm (4.37), *m/e* 404 [76%, (*M*)⁺], 389 [49%, (*M* - CH₃)⁺], and 37 [100%, (*M* - CH₃O)⁺], *m** 344.3 (*m/e* 404 \rightarrow 373). It is very sparingly soluble in chloroform, ethanol, and other organic solvents.

Extraction of Wood of D. ehretioides Wall.—Extraction of the wood (900 g) as for that of *D. buxifolia* gave a pale green solid (5.3 g) which was separated by t.l.c. into (i) plumbagin (132 mg), yellow needles from aqueous methanol, m.p. 76–78° (lit.,¹⁴ 78–79°); (ii) 7-methyljuglone (16 mg), red needles from aqueous methanol, m.p. 126–127° (lit.,¹⁵ 125.5–126.5°); (iii) *diosindigo A* (2 mg); (iv) lupeol (219 mg); (v) mamegakinone (11 mg), red needles from light petroleum (b.p. 80–100°), m.p. 251–253° [lit.,¹⁶ 256° (decomp.)]; (vi) isodiospyrin (6 mg), red needles from light petroleum (b.p. 80–100°), m.p. 224–226° (lit.,¹ 226–228°); and (vii) betulinic acid (646 mg).

Extraction of Wood of D. variegata Kurz.—Extraction of the wood (1.4 kg) as for *D. buxifolia* gave a brown oil (3.1 g) which was separated by t.l.c. into (i) *diosindigo A* (<1 mg); (ii) lupeol (120 mg); (iii) betulin (112 mg); and (iv) mixtures which after acetylation and further t.l.c. gave β -sitosteryl acetate (120 mg), stigmasteryl acetate (81 mg), *O*-acetyl-betulinic acid (110 mg), and *O*-acetyloleanolic acid (108 mg).

Extraction of Heartwood of D. Sanza-Minika A. Chevalier.—Extraction of the heartwood (500 g) as for *D. buxifolia* gave a brown solid (7.3 g), a portion (2 g) of which was separated by t.l.c. into (i) lupeol (360 mg); (ii) a mixture which after acetylation and further t.l.c. gave β -sitosteryl acetate (120 mg) and stigmasteryl acetate (180 mg); (iii) betulin (173 mg); and (iv) betulinic acid (620 mg).

Bergenin.—Further extraction of the *D. Sanza-Minika* heartwood with acetone gave a red-brown oily solid (42 g) which crystallised from methanol to give bergenin monohydrate (19.2 g) as needles, m.p. 129–131° (lit.,¹³ 130°; lit.,¹⁷ ca. 133°) (Found: C, 48.6; H, 5.2%; *M*, 328.0794. Calc. for $C_{14}H_{16}O_9 \cdot H_2O$: C, 48.6; H, 5.2%. Calc. for $C_{14}H_{16}O_9$: *M*, 328.0794), ν_{\max} 3050–2650 (hydrogen-bonded H-O) and 1697 cm^{-1} (hydrogen-bonded lactone C=O), λ_{\max} 221 (log ϵ 4.42) and 276.5 nm (3.91), λ_{infl} 310 nm (log ϵ 3.52), δ [(CD₃)₂SO] 7.02 (1H, s, ArH), 4.98 (1H, d, *J* 10 Hz, ArCH(OR)R), 4.2–3.0 (11H, m, H₂O, ROH, -CH₂- and -CH=), and 3.80 (3H, s, ArOCH₃), *m/e* 328 [33%, (*M*)⁺] and 208 [100%, (*M* - C₄H₈O₄)⁺], *m** 131.9 (*m/e* 328 \rightarrow 208). On heating at 147° and 4 mmHg for 6 h, bergenin monohydrate gave anhydrous bergenin, m.p. 232–234° (lit.,¹³ 234°; lit.,¹⁷ 238°) (Found: C, 51.5; H, 4.7. Calc. for $C_{14}H_{16}O_9$: C, 51.2; H, 4.9%), ν_{\max} 3400–3200 (hydrogen-bonded H-O) and 1720 cm^{-1} (lactone C=O),

δ [(CD₃)₂SO; signals above δ 8.00 only] 9.68 and 8.47 (each 1H, br, ArOH). This was identical with an authentic sample. Acetylation¹³ of bergenin monohydrate gave bergenin penta-acetate which crystallised from methanol as plates, m.p. 208–209° (lit.,¹³ 207–207.5°) (Found: C, 53.6; H, 5.0. Calc. for $C_{24}H_{20}O_{14}$: C, 53.5; H, 4.9%), ν_{\max} 1780 (aryl acetate C=O) and 1743 cm^{-1} (alkyl acetate and lactone C=O), λ_{\max} 257 nm (log ϵ 3.93), λ_{infl} 283 nm (log ϵ 3.43), δ 7.76 (1H, s, ArH), 5.6–3.6 (7H, m, methylene and methine protons), 3.92 (3H, s, ArOCH₃), 2.35 (6H, s, CH₃CO₂Ar), 2.12 (6H, s, CH₃CO₂CH=), and 2.08 (3H, s, CH₃CO₂CH₂).

Diosindigo A Leucoacetate.—A mixture of *diosindigo A* (3 mg), zinc dust (20 mg), anhydrous sodium acetate (20 mg), and acetic anhydride (1 ml) was boiled under reflux for 30 min, and poured into hot water. Extraction with chloroform afforded *diosindigo A leucoacetate*, m.p. 211–212° (from methanol) (Found: *M*, 574.1839. $C_{32}H_{30}O_{10}$ requires *M*, 574.1839), ν_{\max} 1760 cm^{-1} (aryl acetate C=O), λ_{\max} 221 (log ϵ 4.71), 244.5 (4.86), 308 (4.21), 320 (4.16), and 334 nm (4.04), λ_{infl} 256 (log ϵ 4.60) and 296 nm (4.16), δ 8.04br (2H, s, H-5 and H-5'), 7.05br (2H, s, H-7 and H-7'), 6.72 (2H, s, H-3 and H-3'), 3.98 (6H, s, ArOCH₃), 2.53 (6H, s, ArCH₃), 2.36 (6H, s, CH₃CO₂ at C-8 and C-8'), and 2.00 (6H, s, CH₃CO₂ at C-1 and C-1'), *m/e* 574 [2%, (*M*)⁺], 532 [30%, (*M* - CH₂CO)⁺], 490 [23%, (*M* - 2CH₂CO)⁺], 472 (18%, 490 - H₂O), 430 (100%, 490 - H₂O - CH₂CO), 406 (45%, 490 - 2CH₂CO), and 388 (45%, 490 - H₂O - 2CH₂CO).

4-Methoxy-6-methylnaphthalene-1,8-diyl Diacetate (IV; R = Ac).—A mixture of 5-hydroxy-7-methyl-1,4-naphthoquinone¹⁵ (1.0 g), tin(II) chloride dihydrate (1.26 g), and a solution of phosphoryl chloride (1.7 g) in anhydrous methanol (10 ml) was boiled under reflux with stirring for 1 h and poured into *m*-hydrochloric acid (375 ml). The resulting solid was collected, dried, and shaken overnight with acetic anhydride (2.5 ml) and concentrated sulphuric acid (1 drop). The mixture was poured onto ice and the solid product was dried and chromatographed on a column of silica gel. Elution with benzene gave the *diacetate*, which crystallised from ethanol as needles (580 mg), m.p. 122–124° (Found: C, 66.5; H, 5.6. $C_{16}H_{16}O_5$ requires C, 66.65; H, 5.6%), ν_{\max} 1770 cm^{-1} (aryl acetate C=O), λ_{\max} 237 (log ϵ 4.44) and 305 nm (3.83), λ_{infl} 293 (log ϵ 3.75), 316 (3.75), and 332 nm (3.51), δ 2.37 (6H, s, CH₃CO₂Ar), 2.5 (3H, s, CH₃Ar), 3.98 (3H, s, CH₃OAr), 6.77 (1H, d, *J*_o 8 Hz, H-3), 6.84 (1H, d, *J*_o 8 Hz, H-2), 7.00 (1H, *J*_m 1.5 Hz, H-7), and 8.00 (1H, d, *J*_m 1.5 Hz, H-5).

8,8'-Dihydroxy-4,4'-dimethoxy-6,6'-dimethyl-2,2'-binaphthylidene-1,1'-quinone (I; R¹ = Me, R² = OH).—A mixture of the foregoing diacetate (0.5 g) and methanolic 3% potassium hydroxide (20 ml) was boiled under reflux for 30 min under nitrogen, poured into *m*-sulphuric acid (35 ml), and shaken with chloroform (4 \times 35 ml). The dried chloroform solution was warmed on a steam-bath with lead(IV) oxide (3 g) for 5 min, filtered hot, concentrated (to ca. 70 ml), and cooled. The resulting deep blue solid was collected and the filtrate was heated twice more with lead(IV) oxide (2 \times 3 g). The combined blue solids crystallised from chloroform–light petroleum (b.p. 80–100°) to give the 2,2'-binaphthylidene-1,1'-quinone as deep blue needles (180 mg), m.p. 317° (decomp.) (Found: C, 71.0; H, 4.9%; *M*, 404.1263.

¹⁴ L. F. Fieser and J. T. Dunn, *J. Amer. Chem. Soc.*, 1936, **58**, 572.

¹⁵ R. G. Cooke and H. Dowd, *Austral. J. Chem.*, 1953, **6**, 53.

¹⁶ K. Yoshihira, M. Tezuka, and S. Natori, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 2308.

¹⁷ A. E. Tschitschibabin, A. W. Kirssanow, A. J. Korolew, and N. N. Woroschzow, *Annalen*, 1929, **469**, 93.

$C_{24}H_{20}O_6$ requires C, 71.3; H, 5.0%; M , 404.1260). This was identical with diosindigo A.

4,4'-Dimethoxy-6,6'-dimethyl-2,2'-binaphthyl-1,1',8,8'-tetrayl Tetra-acetate (III).—A mixture of the foregoing 1,1'-quinone (50 mg), zinc dust (50 mg), sodium acetate (20 mg), and acetic anhydride (2 ml) was boiled under reflux for 30 min. Hot glacial acetic acid (5 ml) was added, and the mixture was boiled for 5 min, filtered, and poured onto ice. Extraction with chloroform gave the *tetra-acetate* (70 mg), which crystallised from ethanol as needles, m.p. 210—212°

(Found: C, 66.6; H, 5.2. $C_{32}H_{30}O_{10}$ requires C, 66.9; H, 5.25%). This was identical with diosindigo A leucoacetate.

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